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GENETIC STRUCTURE AND EVOLUTION OF A FIRE ANT HYBRID ZONE

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Abstract.—Two introduced fire ants, *Solenopsis invicta* and *S. richteri*, hybridize over an extensive area in the United States spanning central Mississippi, Alabama, and western Georgia. We studied a portion of this hybrid zone in northwestern Mississippi in detail by sampling ants at many sites along two transects extending across the zone and examining gene frequency and size distributions at a large number of genetic and morphological markers. The distributional patterns at these markers are most consistent with the mosaic hybrid zone model, whereby the distribution of various fire ant genotypes is determined initially by the historical patterns of colonization of newly available habitats. However, these distributional patterns probably do not reflect the equilibrium state of interactions because of the very recent secondary contact of the species (< 60 yr) and the dynamic nature of available nesting habitats in this area. Our data suggest that, with prolonged contact and interaction, differential fitness of various hybrid genotypes due to intrinsic and extrinsic selective factors is important in structuring the hybrid zone. For instance, consistent differential introgression of morphological and genetic markers, combined with previous evidence of differences in developmental stability among genotypes, suggest reduced fitness of hybrids relative to parentals due to intrinsic selection (as may be caused by breakup of parental gene complexes). Furthermore, marked reductions in the occurrence of parental-like hybrids in areas where the similar parental species is common suggest reduced fitness of these parental-like hybrids in competition with the parentals (i.e., extrinsic selection). Because the relative roles of such deterministic as well as stochastic forces apparently vary both spatially and temporally, the eventual distribution of the various fire ant genotypes and the fate of the hybrid zone in the United States is difficult to predict.

Key words.—Fire ants, hybrid fitness, hybridization, mosaic hybrid zone, *Solenopsis invicta*, *Solenopsis richteri*.

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Natural hybrid zones are rich sources of information for evolutionary genetic studies (Harrison 1990, 1993). Studies of hybrid zones can provide insights into the effects of introgression on the maintenance of genetic distinctiveness, into the genetic architecture and functioning of reproductive barriers to gene exchange, and into the genetic and ecological nature of species differences (e.g., Barton and Hewitt 1985, 1989; Kocher and Sage 1986; Szymura and Barton 1986, 1991; Barton and Gale 1993; Harrison 1990, 1991, 1993). For example, comparisons of patterns of introgression and estimation of single-locus and linkage disequilibria within a hybrid zone can aid in determining the relative fitness of hybrid genotypes (Rand and Harrison 1989; Harrison 1990, 1991, 1993; Barton and Gale 1993; Arnold and Hodges 1995). Determination of hybrid fitness is key to understanding the eventual distribution and evolutionary fate of the two parental species and their hybrids. If hybrids are less fit than both parental species, then hybridization may represent a culminating step in speciation by leading to the evolution of premating barriers to interbreeding (i.e., reinforcement; Dobzhansky 1940, 1941, 1970). Alternatively, if some hybrid genotypes are more fit than the parental species in some environments, then hybridization may lead directly to the origin of hybrid species, thus serving as a source of evolutionary innovation through the production of novel genotypes (Whitham et al. 1991; Arnold 1992; Harrison 1993; Bullini 1994).

Studies of a hybrid zone formed between two introduced fire ants, *Solenopsis invicta* and *Solenopsis richteri,* in the United States hold promise for addressing some of these issues because the history of formation of the zone is known, the reproductive and dispersal biology of these species is well understood, and information on the population and molecular genetics of these ants is increasing (Markin et al. 1971; Lofgren 1986; Ross and Trager 1990; Ross et al. 1987a,b, 1988, 1993; Shoemaker et al. 1994). These two species, members of a large group of ants native to South America included in the *Solenopsis saevissima* species complex (Trager 1991), apparently are parapatric and do not hybridize extensively in their native habitats (Ross and Trager 1990). They were introduced into Mobile, Alabama, at separate dates earlier in this century (*S. richteri* around 1918 and *S. invicta* around 1935) and, collectively, have spread to occupy most of the southeastern United States, from central Texas to southern Virginia (Culpepper 1953; Buren et al. 1974; Lofgren 1986). Hybridization between the two species in the USA occurs over an extensive area from western Georgia through Alabama to central Mississippi (Ross et al. 1987a; Diffie et al. 1988; Shoemaker et al. 1994). Thus, the fire ant hybrid zone clearly has formed as a result of secondary contact and represents one of the few well-documented examples of an animal hybrid zone forming in historical times (Vander Meer et al. 1985; Ross et al. 1987a; Echelle and Connor 1989; Shoemaker et al. 1994).

We recently determined the distribution of *S. invicta*, *S. richteri*, and their hybrids on a macrogeographic scale in the southeastern United States using allozyme and DNA markers (Shoemaker et al. 1994). One significant pattern that emerged from this study is that the width of the hybrid zone varies considerably from one geographic locality to another. In northwestern Mississippi, the western limit of the joint ranges of the two species and their hybrids, the ranges of the two parental species actually may come into contact, whereas in regions farther east the two parental species are separated by hybrid populations extending over more than 100 km (Shoemaker et al. 1994; Fig. 1). The narrow width of the hybrid zone in the west may indicate more recent colonization and

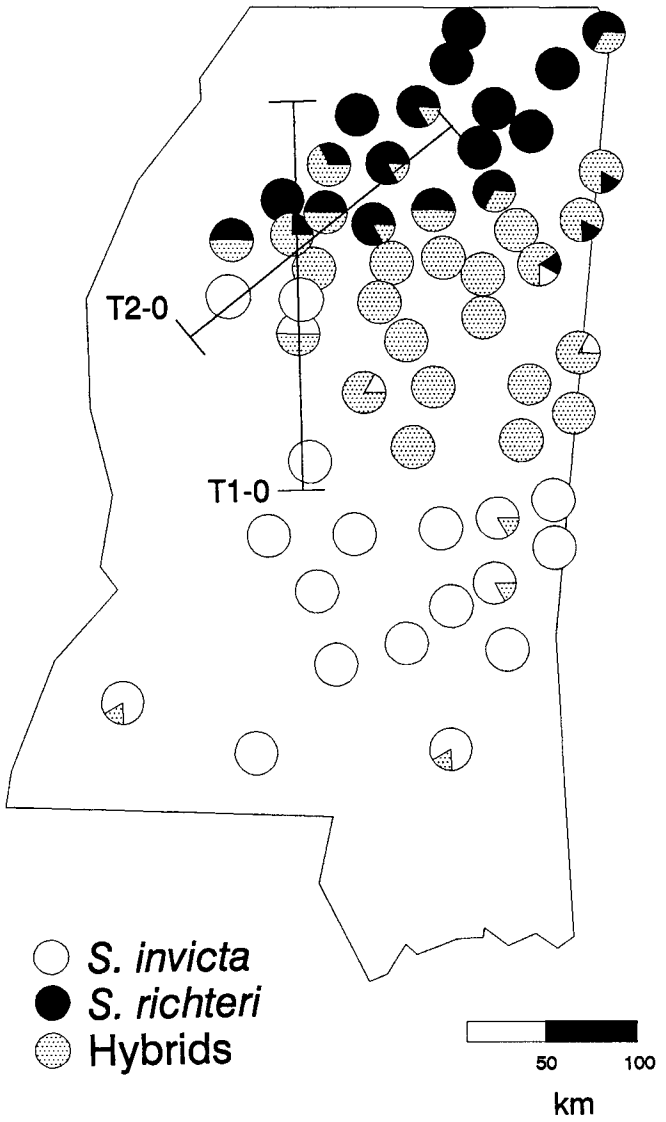


FIG. 1. Distribution of *Solenopsis invicta*, *S. richteri*, and hybrid colonies in Mississippi (data from Shoemaker et al. 1994). Pie diagrams represent the proportion of colonies of each type at each sampling site. The two sampling transects for the present study are designated T1 and T2. Sites T1-0 and T2-0 represent the southernmost collection sites on each transect. See also Appendices 2 and 3.

contact between the parental forms compared to regions farther east.

In the present study we examine this region in northwestern Mississippi in more detail by using a large number and variety of genetic markers and a high-resolution sampling scheme. We sampled ants along two transects with different orientations that span regions of the hybrid zone of different widths to determine whether consistent genetic patterns exist for different classes of markers in different regions of this zone. This was important because factors that influence the genetic structure of a hybrid zone (i.e., the history and sequence of colonization events, structure and availability of suitable habitats, and fitness of individuals of different genetic constitution) may vary both spatially and temporally and may affect various elements of the genome differently. Thus, our main goal is to infer the evolutionary processes important in determining the structure and eventual fate of the fire ant hybrid zone by integrating data on patterns of genetic variation along these two transects with previous data on the fitness of the two species and their hybrids (Ross et al. 1987a; Ross and Robertson 1990; Shoemaker et al. 1994).

MATERIALS AND METHODS

Sample Collection

Data on the distributions of *S. invicta*, *S. richteri*, and their hybrids at macrogeographic scales aided in deciding where genetic studies at microgeographic scales should be conducted (Shoemaker et al. 1994). We focused these microgeographic analyses along two transects extending across the hybrid zone from one parental species to the other in northwestern Mississippi (transects T1 and T2, Fig. 1). We chose this area for detailed study because it apparently represents an area of recent contact and interaction between the two species, as suggested by the known history of the spread of fire ants, the apparent narrow width of the hybrid zone, and the patchy distribution of fire ants in this region compared to more easterly regions (Buren et al. 1974; Lofgren 1986; Shoemaker et al. 1994). Because of the recent contact of the species in this area, we have an unique opportunity to "view" the evolutionary forces important in structuring a hybrid zone early in the process of hybridization. Also, our two transects may record somewhat different histories and durations of interactions between the two species (because of their different locations and orientations), with the southwesternmost part of transect T2 probably lying in the area of most recent contact.

We collected fire ants from 44 sites distributed relatively evenly along the two transects. Transect T1 is approximately 210 km long and lies along Interstate Highway 55, extending from Canton, Mississippi northward to Sardis, Mississippi (Madison, Yazoo, Holmes, Carroll, Montgomery, Grenada, Yalobusha, and Panola Counties); transect T2 is approximately 190 km long and lies along State Highways 7 and 9, extending from Morgan, Mississipppi northeastward to Ellistown, Mississippi (Leflore, Carroll, Grenada, Yalobusha, Calhoun, Pontotoc, and Union Counties; see Fig. 1 and Appendix 1 for specific site locations). We collected a single adult winged queen from each of 10–44 colonies at each site ($n = 1425$ queens; $\bar{x} = 32.4$ queens per site). We collected only a single queen per colony because fire ant colonies comprise families and, hence, the genotypes of colony-mates may not always be independent (Ross and Fletcher 1985; Ross 1993). Collected queens were placed in liquid nitrogen in the field and later stored in a freezer at −70°C pending genetic and morphological analyses.

Morphological Analyses

We measured five continuous (metric) characters in the collected queens because previous analyses demonstrated that the parental species differ significantly in the size of each (Ross and Robertson 1990). These five characters were the radial cell, cell radius one, and medio-cubital crossvein on the forewing; the metathoracic femur; and the antennal scape

(see Ross and Robertson [1990] for descriptions of the characters). The length of each left-side character in each collected queen was determined using the ocular micrometer of a dissection microscope. Repeat measurements were made the following day to quantify measurement error.

Individual character sizes at each site were jackknifed (e.g., Crowley 1992) to give a mean value and variance; 95% confidence intervals about the mean character values were constructed from the variances assuming a *t*-distribution. We compared the distributions of character sizes between the two species using a nested analysis of variance (ANOVA), with the sources of variance including measurement error and between-individual variation within each species as well as between-species variation. For these analyses, we combined all sites that contained only pure parental colonies (as defined by the diagnostic genetic markers).

Allozyme Analyses

Electrophoresis of allozymes was conducted on horizontal starch gels as outlined by Shoemaker et al. (1992). A total of seven informative allozyme loci were scored from the thoraces of each of the 1425 queens collected. Previous studies have shown that these seven loci exhibit Mendelian patterns of inheritance and are informative in distinguishing *S. invicta* and *S. richteri* in the United States (Ross et al. 1987a,b; Ross and Robertson 1990; Shoemaker et al. 1994). Two of these loci (*Est-2* and *Gpi*) are diagnostic for the two species (i.e., alternate alleles are fixed in each species; cf. Ayala and Powell 1972), whereas at the other five loci alternate alleles are almost fixed in each species (*Aat-2*, *Odh*) or one of the species possesses an allele at relatively high frequency that is absent in the other species (*Est-4*, *G3pdh-1*, and *Pgm-1*).

RAPD DNA Analyses

We used only the queens collected along transect T1 for our DNA analyses. Total genomic DNA was isolated from the gasters (abdomens) for this subset of queens as described by Shoemaker et al. (1994). DNA from each queen was amplified via the polymerase chain reaction to determine genotype at the single codominant marker *UBC 105*. This marker previously was shown to be inherited in Mendelian fashion and is diagnostic in distinguishing *S. invicta* and *S. richteri* in the United States (Shoemaker et al. 1994). Protocols for reaction mixtures, PCR conditions, and gel electrophoresis followed those of Shoemaker et al. (1994). The use of only the gasters for the DNA analyses and thoraces for the allozyme analyses allowed us to reconstruct complete multilocus genotypes of each individual collected along transect T1.

Genetic Data Analyses

We estimated the frequencies of each *S. invicta* allele for each allozyme and DNA locus at each site. The 95% confidence intervals about each frequency estimate were generated by drawing 1000 bootstrap samples from the original data sets, estimating the allele frequencies for each of the bootstrap samples, and eliminating the 25 extreme low and 25 extreme high values from the ordered array of sample estimates (Weir 1990).

The observed genotype proportions for each locus at each site were compared to those expected under Hardy-Weinberg equilibrium (HWE) using the program GENEPOP (Raymond and Rousset 1995). This program tests for single-locus disequilibrium using Fisher's Exact Test (Louis and Dempster 1987; Weir 1990). We used a sequential Bonferroni procedure (Hochberg 1988) to evaluate the statistical significance of each test because of the large number conducted.

Pairwise composite linkage disequilibria were estimated for each pair of variable loci at each site using a program written by D. Zaykin and B. Weir (pers. comm.). The program uses the Markov chain method to estimate the exact probability that a given pair of loci are in linkage equilibrium, that is, that alleles and genotypes at the two loci are randomly associated. This program corrects for departures of genotype proportions from HWE at single loci before calculating the exact probability of linkage equilibrium. However, any existing higher-order disequilibria (associations among three or more loci) are not eliminated from these estimates. The 95% confidence intervals about the proportions of cases of significant disequilibria were generated by drawing 1000 bootstrap samples from the original data sets, estimating the proportions of significant disequilibria in each of the bootstrap samples, and eliminating the 25 extreme low and 25 extreme high values from the ordered array of sample estimates (e.g., Crowley 1992). Also, we used a sequential Bonferroni procedure to evaluate the statistical significance of each exact probability of linkage disequilibrium.

Allele frequencies at each locus and sizes of each morphological character at each site were compared to determine if any patterns of coincidence, concordance, and clinality were apparent both within and among the different classes of markers. For these comparisons, we transformed the frequencies of the common *S. invicta* alleles and the sizes of each of the five morphological characters to range from zero to one, with zero representing pure *S. richteri* and one representing pure *S. invicta*. Visual inspection of these data suggested parallel patterns in the changeovers from the characters of one species to those of the other on a site by site basis both within and among the three classes of markers along both transects (i.e., the character transitions through the hybrid zone appeared *coincident* among all markers). Therefore, we used standard regression techniques on the transformed values for each pair of markers to determine whether the character transitions also were *concordant*, that is, whether the changeovers from the characters of one species to those of the other occurred at similar rates among the different markers. These analyses consisted of constructing bivariate plots for all pairs of markers and determining if the slopes of the regression lines drawn through these values differed significantly from a slope of one, which would represent complete concordance between a given marker pair (identical rates of change through the zone). We excluded *Est-4* and *G3pdh-1* from these analyses because the small ranges of observed values for the allele frequencies at these two loci yield unreliable values when transformed. We used a Bonferroni procedure to evaluate the statistical significance of departures of regression line slopes from one, adjusting

the alpha level by dividing it by the total number of tests performed (Hochberg 1988).

We compared the observed numbers of pure parental and hybrid individuals at each site with the numbers expected under HWE. These comparisons allowed us to determine whether there was a deficiency of hybrids at a given site relative to the proportion predicted under the assumptions of no selection, no large-scale immigration, and random mating explicit in the Hardy-Weinberg Law. The observed number of hybrid or parental ants was determined by inspecting the multilocus genotype of each ant using all eight informative loci (seven for transect T2). Thus hybrids were identified by their possession of multilocus genotypes inconsistent with those of either parental species. We generated the expected numbers of hybrid and parental ants at each site using only the five most informative loci—*Aat-2*, *Est-2*, *Gpi*, *Odh*, and *UBC 105* (only the four allozyme loci for transect T2)—and assuming independent assortment of these loci and HWE. Therefore, our estimates are slightly biased toward a lower number of *expected* hybrids at a given site than would be the case had we employed all of the markers. We generated 95% confidence intervals around our estimates of the observed numbers of hybrids by drawing 1000 bootstrap samples from the original sets of queen identifications at each site, estimating the proportion of hybrids in each of these samples, and eliminating the 25 high and 25 low values from the ordered arrays of sample estimates.

We calculated a hybrid index score for each queen at each site using genotypes at the three diagnostic markers (two in the case of transect T2). A one was added to each queen's score for each *S. richteri* allele that she possessed, so that a score of zero represents pure *S. invicta*, a score of six (or four in the case of transect T2) represents pure *S. richteri*, and intermediate scores represent hybrids. The scores were used to determine if there was a deficiency of hybrids at any of the sites and, if so, to determine if this was the result of underrepresentation of any particular type of hybrids (*S. invicta*-like, *S. richteri*-like, or intermediate hybrids). Expected distributions of scores at each site were calculated from the observed allele frequencies at the diagnostic loci under the assumptions of independent assortment of these loci and HWE. We generated 95% confidence intervals for each of the observed hybrid index scores at each site by drawing 1000 bootstrap samples from our original data set of hybrid index scores, estimating the number of individuals with each score at each site in each bootstrap sample, and eliminating the 25 high and 25 low values from the ordered array of sample estimates.

Results

Morphological Character Sizes

Mean character sizes were significantly greater in *S. richteri* than in *S. invicta* for all five characters (all $P < 0.05$), confirming the results of Ross and Robertson (1990). Measurement error was not a significant factor contributing to the observed size variance; an ANOVA demonstrated that between-individual variation within species explains 44–79% of the observed variance, whereas measurement error explains less than 1% of the variance in all cases. The significant differences in sizes of these characters between the two species means that the characters constitute a useful class of markers for studying the genetic structure of the hybrid zone.

Single Locus Disequilibria

We observed 46 deviations from HWE significant at the 0.05 level (representing 18% of the 246 tests performed), with all but one of these departures due to a deficiency of heterozygotes (Fig. 2; Appendices 2 and 3). These departures were distributed among many individual sites along the two transects. We observed eight significant departures from HWE (3%) when the significance levels were adjusted using a Bonferroni procedure (Fig. 2; Appendices 2 and 3), all due to heterozygote deficiencies. The true extent of significant heterozygote deficiency in this fire ant hybrid zone most likely lies somewhere between our two estimates. This is because the Bonferonni procedure, which sequentially adjusts the alpha level to correct for Type I errors when multiple tests are performed (Hochberg 1988), becomes conservative with large numbers of tests, leading to an inflation of Type II errors.

We also tested for departures of genotype proportions from HWE after eliminating from the analyses all individuals with pure parental genotypes (determined by inspecting the multilocus genotypes of each) to determine whether the previously observed departures could be explained simply by the presence of pure parental individuals within the hybrid zone. Only 5% (11 out of 210) of these genotype proportions deviated significantly from HWE without correcting for multiple tests, whereas no significant departures from HWE were observed using the sequential Bonferroni procedure. Thus, there is no evidence for persistent single-locus disequilibrium among individuals of mixed ancestry in the portion of the fire ant hybrid zone we studied.

Linkage Disequilbria

Estimates of composite genotypic linkage disequilibria revealed significant disequilibria that involve virtually all pairs of loci and that persist even when significance levels are adjusted using the Bonferroni procedure (Fig. 3). These significant disequilibria are distributed among many different sites occurring along both transects. Linkage disequilibria were not significant for five pairs of loci that included in each case either *Est-4*, *G3pdh-1*, or *Pgm-1*. This is not unexpected given that the two species share relatively common alleles at these three loci, making it difficult to distinguish recombinant genotypes from nonrecombinant (pure species) genotypes.

We calculated pairwise linkage disequilibria again after eliminating all individuals with pure parental multilocus genotypes. Only 5% (43 of 691) of these comparisons were significant (none were significant when the alpha levels were adjusted using a sequential Bonferroni procedure). This demonstrates that, as in the case of single-locus disequilibrium, all significant linkage disequilibria can be explained by the presence of individuals with pure parental genotypes within the hybrid zone. We also performed these analyses by combining adjacent sites with similar allele frequencies to increase our sample sizes for added statistical power. Nine percent (12 of 140) of these comparisons yielded significant linkage disequilibria. However, three of the significant dis-

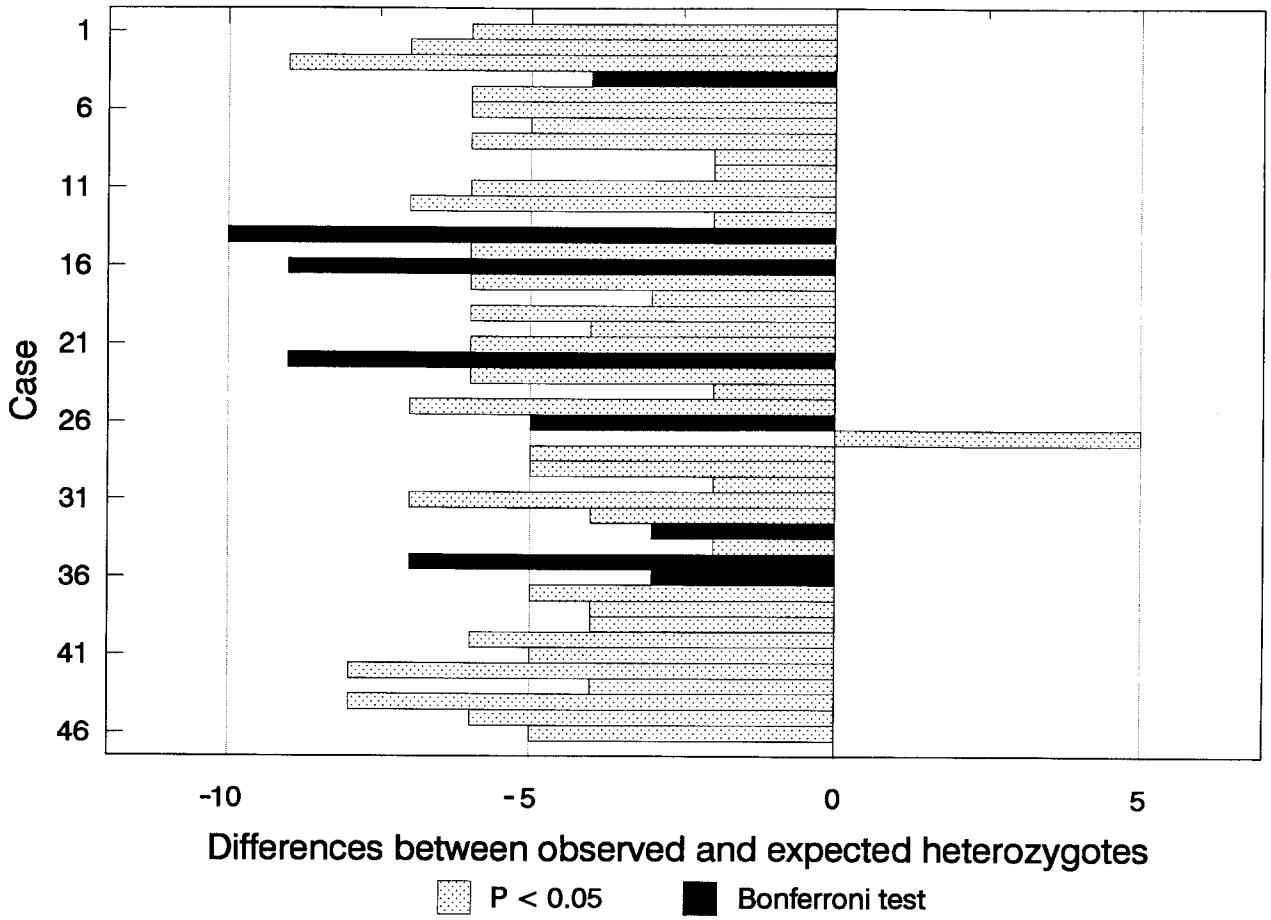


FIG. 2. Differences between observed and expected numbers of heterozygotes for each of 46 comparisons in which the observed genotype proportions departed significantly from the proportion expected under Hardy-Weinberg equilibrium at the 0.05 level. Negative and positive values indicate deficiencies and excesses, respectively, of observed heterozygotes. Black bars represent the eight departures that remain significant using the sequential Bonferroni procedure to adjust significance levels.

equilibria were between two allozyme loci, *Est-2* and *Est-4*, that are linked (K. G. Ross and D. D. Shoemaker, unpubl. data). When comparisons between these two loci were excluded, we observed only nine significant instances of linkage disequilibrium in 133 tests (7%; 2 of 133 using the sequential Bonferroni procedure).

Coincidence, Concordance, and Clinality of Markers

Visual inspection of the relative frequencies of *S. invicta* alleles at the allozyme and DNA loci and of the relative sizes of the morphological characters suggests generally coincident changes among markers of all three of these classes on a site by site basis along both study transects (Fig. 4). That is, parallel patterns of increase and decrease in the allele frequencies and character sizes are apparent at most sites.

Concordance between all pairs of markers was studied by examining slopes of the regression lines drawn for the bivariate plots of the standardized allele frequencies and character values. The slopes generally are statistically indistinguishable from one (complete concordance) when genetic markers are compared to one another or morphological markers are compared to one another, indicating that the changeovers from the characters of one species to those of the other occurred at the same rate within each of these two broad types of markers (Table 1). The only significant departure from concordance between markers of a single type using a Bonferroni procedure involved comparisons between the allozyme loci *Odh* and *Pgm-1* along transect T2. This departure may represent true discordance, but more likely results from scaling error associated with the narrow range of allele frequency differences between the two species at *Pgm-1*. The fact that these loci were concordant along transect T1 argues further that the lack of concordance along transect T2 is an artifact of this error.

In contrast to the strong concordance among markers of a given type, the transitional patterns showed evidence of consistent discordance between the genetic and morphological markers. For instance, the slopes of the regression lines when allele frequencies were plotted as the dependent variable against morphological character values were in all 55 cases less than one (binomial probability that this pattern arises by chance, $P < 3.0 \times 10^{-17}$). Indeed, 80% of these regression line slopes were significantly less than one when no corrections for multiple tests were made and 15% remained sig-

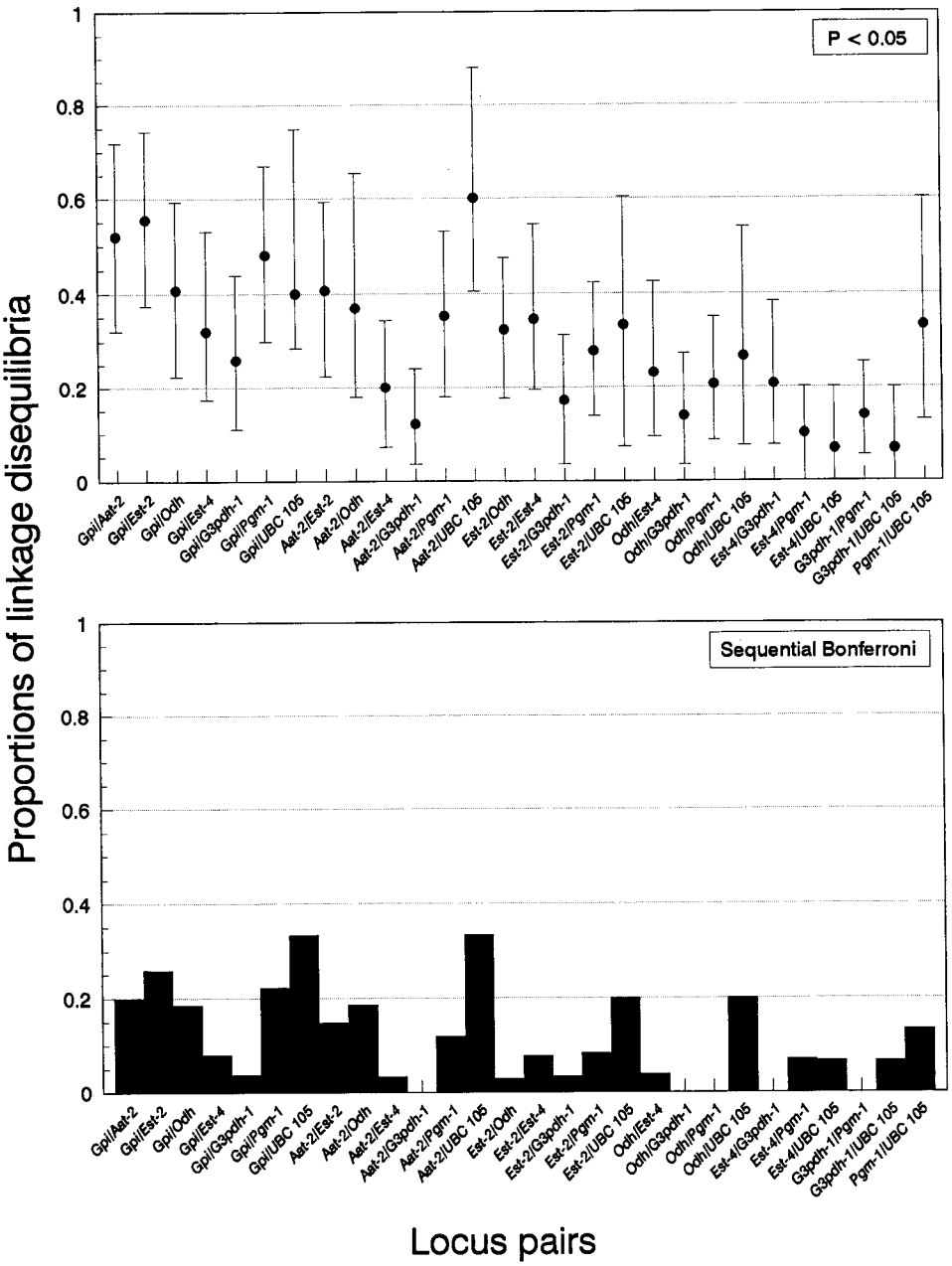


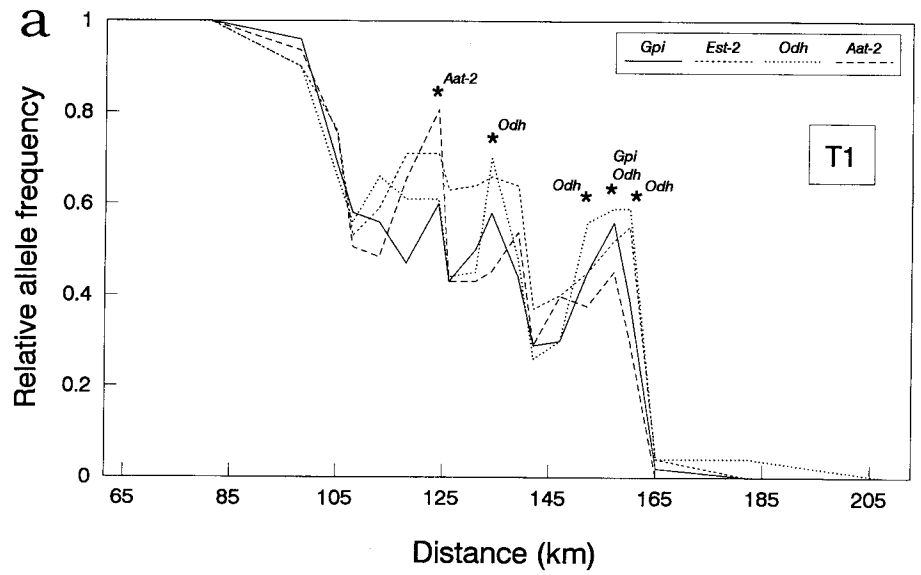
FIG. 3. Proportions of significant genotypic linkage disequilibria among all possible pairwise comparisons of loci along transects T1 and T2. The data represent the significant proportions of departures both without (upper panel) and with (lower panel) significance levels adjusted using a sequential Bonferroni procedure. Bars around the values in upper panel represent 95% confidence intervals about the proportions of cases of significant disequilibrium.

nificant when the significance levels were adjusted using a Bonferroni procedure. These results suggest that the changeover in morphology from one species to the other along both transects is more abrupt than the changeover in allele frequencies at single Mendelian genes.

We did not observe a smooth clinal transition from the characters of one species to those of the other along either transect for any of the markers (Fig. 4). For instance, we observed significant reversals in the changeover from *S. invicta* to *S. richteri* alleles (indicated by a significant increase in *S. invicta* allele frequencies relative to previous sites) for at least one locus at five and at four sites along transects T1 and T2, respectively (Fig. 4). Similar pattern reversals are evident for the morphological markers. The lack of clinality is especially apparent along the southwesternmost half of transect T2, where there are abrupt swings from the characters typical of one species to those typical of the other, with four sites exhibiting significant allele frequency reversals at most loci (Fig. 4).

Proportions of Hybrids

We observed deficiencies of hybrids relative to the frequencies expected under HWE at many sites along both tran-


a
Relative allele frequency
Gpi
Est-2
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T1
Aat-2
Odh
Odh
Gpi
Odh
Odh
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0.2
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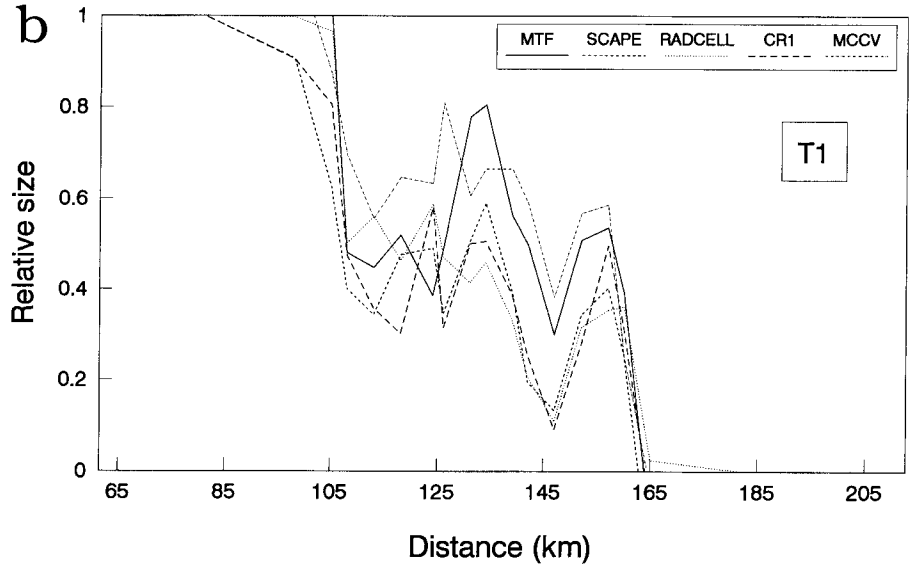
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Relative size
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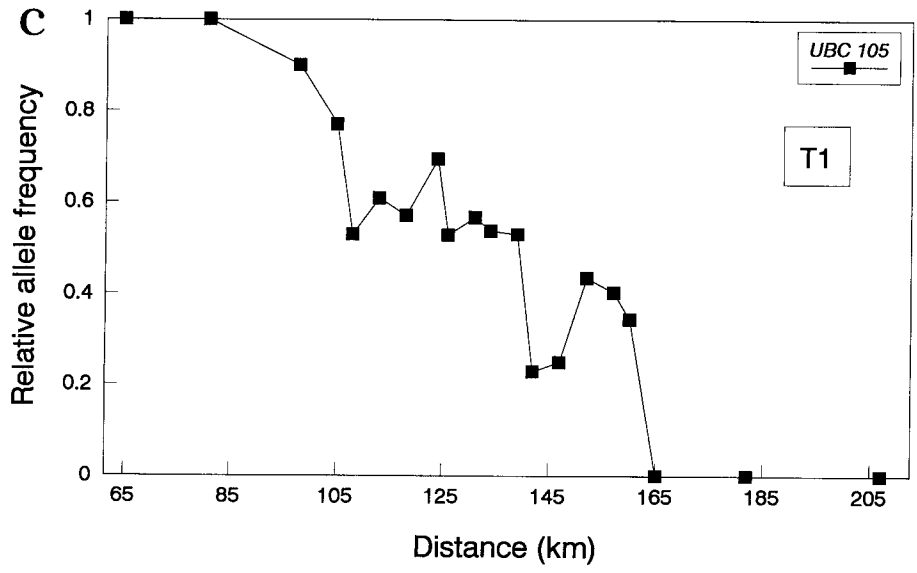
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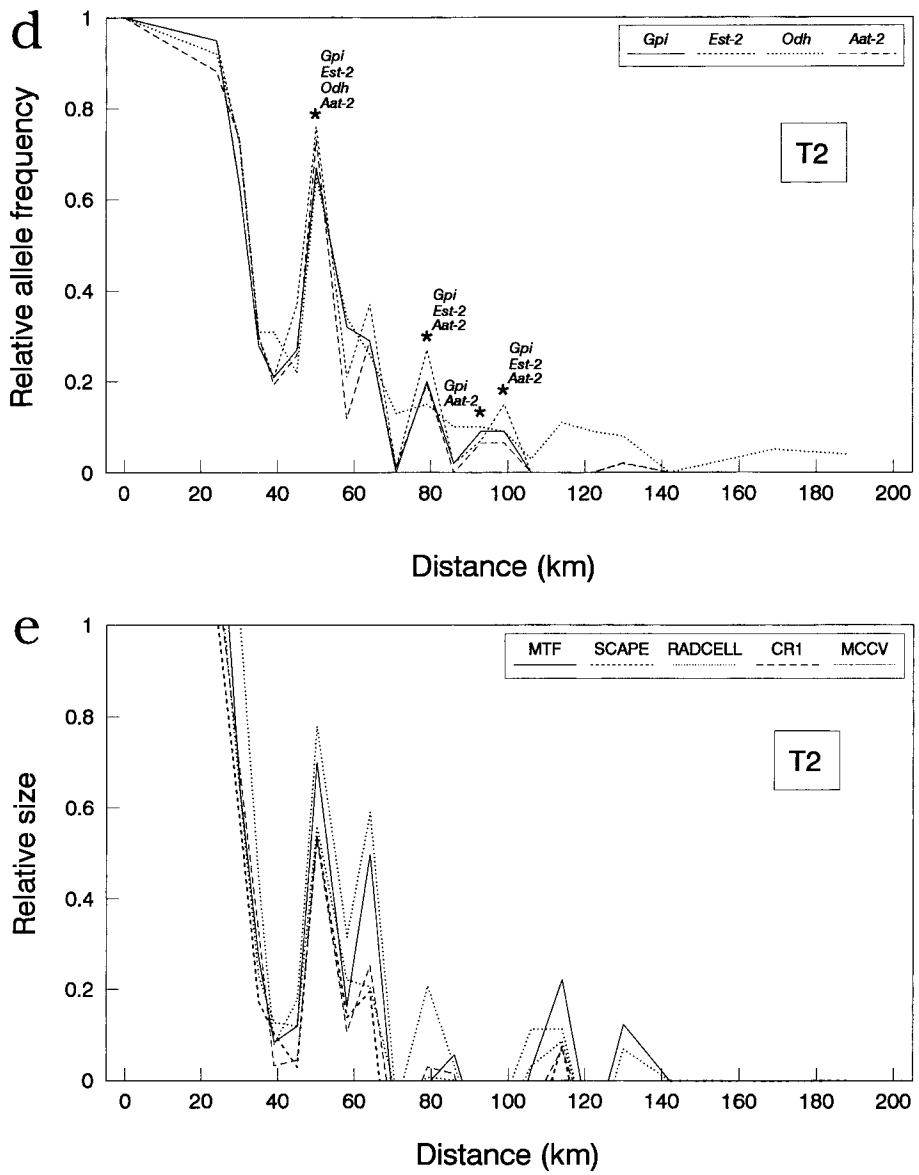

FIG. 4. Relative frequencies of *Solenopsis invicta* alleles at the allozyme and DNA loci and relative sizes of five morphological characters along transects T1 (a–c) and T2 (d–e). Only allozyme loci with fixed or nearly fixed allelic differences between the parental species are considered. Asterisks indicate sites where allele frequencies at one or several loci are significantly greater than frequencies at previous sites, in opposition to the general trend of a changeover from *S. invicta* alleles in the south to *S. richteri* alleles in the north along the transects. Distance 65 km on transect T1 coincides with the location of T1-65 (this site is 65 km north of the southernmost site on this transect; this site and all sites south include only pure *S. invicta*; see Appendix 2); distance 0 km on transect T2 coincides with the location of Site T2-0, the southernmost site on this transect (which also includes only pure *S. invicta*; Appendix 3); (a, d) allozymes; (b, e) morphological characters; (c) RAPD DNA marker *UBC 105*.

sects. For example, of 17 sites along transect T1 that were not populated by only one parental species, hybrids were unexpectedly rare at 10 (59%). Similarly, we observed a deficiency of hybrids at 10 of 19 appropriate sites (53%) along transect T2. This consistent underrepresentation of hybrids throughout the study area suggests that the ants inhabiting the area do not constitute an equilibrium population meeting the assumptions of the Hardy-Weinberg Law. Rather, reduced fitness of individuals of mixed ancestry, positive assortative mating, or lack of equilibrium conditions due to continual immigration or a short time of interaction between the species seem to be important factors determining the genetic structure of the hybrid zone.

Hybrid Index Scores

We constructed a hybrid index score for each queen from the genotypes at the diagnostic loci to determine if the underrepresentation of hybrids in the zone could be explained by the absence of particular classes of hybrids (i.e., *S. invicta*-like hybrids, *S. richteri*-like hybrids, or intermediate hybrids). We observed deficiencies of hybrids in one or more of these

TABLE 1. Slopes of regression lines fitted to bivariate plots for each pair of markers at each site along transects T1 (above diagonal) and T2 (below diagonal). For comparisons between markers of different types, the genetic markers are the dependent variables. Comparisons that were significant using a Bonferroni procedure are indicated in bold with asterisks. Upper right and lower left quadrants (boxes) represent comparisons between the genetic and the morphological markers. Genetic markers are italicized, whereas the morphological markers are in uppercase, unitalicized letters.

	Aat-2	*Est-2*	*Gpi*	*Odh*	*Pgm-1*	*UBC 105*	CR1	MCCV	MTF	RADCELL	SCAPE
Aat-2	—	1.027	0.977	0.888	0.874	0.943	0.787	0.725	0.560	0.740	0.819
Est-2	0.959	—	0.863	0.855	0.785	0.848	0.697	0.650	**0.566***	0.642	0.770
Gpi	1.005	1.047	—	0.937	0.880	0.863	0.794	0.686	0.603	0.710	0.808
Odh	1.072	1.092	1.053	—	0.806	0.798	0.691	0.571	0.530	0.640	0.751
Pgm-1	0.855	0.881	0.824	**0.759***	—	0.876	0.822	0.698	0.668	0.721	0.828
UBC 105	—	—	—	—	—	—	0.819	0.744	0.612	0.786	0.856
CR1	0.755	0.764	0.735	0.693	0.874	—	—	0.789	0.744	0.859	0.939
MCCV	**0.645***	**0.662***	**0.628***	0.579	0.744	—	0.822	—	0.774	0.811	0.961
MTF	**0.654***	0.669	**0.643***	0.597	0.775	—	0.874	1.025	—	0.901	1.111
RADCELL	0.820	0.838	0.816	**0.765***	0.957	—	1.053	1.232	1.165	—	0.941
SCAPE	0.732	0.746	0.723	**0.683***	0.860	—	0.958	1.114	1.067	0.883	—

classes, and usually corresponding excesses of one or both parental species, at 12 of the 16 sites along transect T1 and 8 of the 18 sites along transect T2 (Appendices 4 and 5). Overall, these significant deficiencies appear to be distributed rather evenly among the various classes of hybrids, suggesting that the paucity of individuals of mixed ancestry does not result from a general lack of only *S. invicta*-like hybrids, *S. richteri*-like hybrids, or genetically intermediate individuals. However, underrepresentation of the different hybrid classes appears not to be uniform in its distribution along each transect. For example, all of the sites with deficiencies of *S. invicta*-like hybrids have excesses of pure *S. invicta* and are located near the range of this parental species (Appendices 4 and 5). This same pattern holds generally for *S. richteri*-like hybrids; the sites where such hybrids are lacking usually contain an excess of pure *S. richteri* and tend to occur close to the range of this parental species.

DISCUSSION

Genetic Structure of the Fire Ant Hybrid Zone

We observed somewhat different transitional distributions in allele frequencies and character sizes between our two transects through the fire ant hybrid zone. Rather gradual and persistent changeovers from alleles and character sizes characteristic of one species to those of the other were observed along transect T1, with relatively modest reversals in these changes. Along transect T2, however, striking reversals occurred in the transitions of allele frequencies and character sizes between the two pure parental areas, particularly along the southwestern half of this transect. This pattern along transect T2 is indicative of a mosaic distribution of pure parentals and hybrids of varying genetic constitution in this far western region of the hybrid zone. Particularly notable are pure populations of *S. richteri* that are nested between sites containing mostly hybrids near the center of transect T2 (Fig. 4).

It is tempting to explain the different transitional patterns along our two transects by recourse to opposing models of hybridization that emphasize the importance of different forces. For example, the patterns along transect T1 might be explained on a broad scale by the tension zone model of hybridization (Key 1968; Barton and Hewitt 1985). Under this model, a hybrid zone is maintained by the joint effects of dispersal of parental genotypes into the hybrid zone and intrinsic selection acting against hybrid genotypes because of their reduced fitness compared to the parentals. A balance between these two forces leads to the formation of stable clines, with the widths of these clines determined by the magnitudes of dispersal and selection (Key 1968; Barton and Hewitt 1985). Environmental (extrinsic) selection is not considered important in maintaining or structuring such hybrid zones. Consistent with this model, we observed a relatively gradual transition in allele frequencies and character sizes along transect T1 on a large scale.

However, closer scrutiny of the transitional patterns along transect T1 reveals that these are not consistent with the tension zone model. We observed significant reversals in the transitions of one or more genetic markers at five sites along this transect rather than persistent changeovers (Fig. 4). Such patterns can be explained more readily by a mosaic model of hybridization, as can those along transect T2 (Harrison 1986; Howard 1986; Rand and Harrison 1989). Mosaic hybrid zones are created when the parental species survive best in distinct habitats that are distributed in mosaic fashion or, as is probable in our case, when they occupy disturbed habitats that are distributed patchily and are formed continually (Harrison 1986; Howard 1986; Rand and Harrison 1989; Harrison 1990). In this latter case, a mosaic distribution of the two species and their hybrids, such as we observed along our transects, reflects mostly the historical sequence of colonization events of newly available, suitable patches. This model differs from the tension zone model of hybridization most conspicuously in that the environment (extrinsic selection) is ascribed importance in structuring the distribution of the parental species and their hybrids. However, reduced hybrid fitness, a necessary component of the tension zone model of hybridization, is an important assumption of the mosaic model as well (Harrison 1986, 1990; Rand and Harrison 1989).

The genetic structure of the fire ant hybrid zone and the differences observed between the two study transects may

be explained by the patchy distribution of suitable fire ant habitat coupled with recent colonization of this area (Buren et al. 1974). Fire ants occupy disturbed or open habitats such as pastures or roadsides (Markin et al. 1973; Lofgren et al. 1975; Lofgren 1986). Such habitats tend to be patchily distributed within the environment and are created and lost in an unpredictable fashion. The initial genetic character of fire ants in such patches is determined by the colonists that arrive first, the identity of which has a large stochastic element depending on the wind strength and direction during mating flights and the proximity and genetic makeup of ants in neighboring habitats (Markin et al. 1972, 1973). Thus, we might expect to observe a mosaic distribution that largely reflects the stochastic elements of colonization in the early stages of hybridization, regardless of the eventual fate of the various genotypes and final structure of the zone. The different patterns of allele frequency and character size changeover along our two transects most likely reflect different histories of colonization and interaction of fire ants in these two areas of the hybrid zone, with the southwestern portion of transect T2 representing the period of briefest interaction between the genetically distinct parental types (Shoemaker et al. 1994). The history of the movement of the two species and their hybrids (Buren et al. 1974; Lofgren 1986), the low colony densities and comparatively narrow width of the hybrid zone in this area (D. D. Shoemaker and K. G. Ross, pers. obs.; Shoemaker et al. 1994), and the striking mosaicism and abrupt character transitions that we observed all suggest that this is an area of very recent contact between *S. invicta* and *S. richteri*.

We might expect a quite different structure in areas of prolonged interaction between the two species and their hybrids. This is because factors such as the competitive abilities or overall fitnesses of the various genotypes become more important in determining the genetic structure of the hybrid zone with increased time of interaction. The patterns we observed along transect T1 may represent a more advanced stage of hybridization that has a relatively long history of being influenced by such factors. Thus, the diminished mosaicism and more clinal transition in allele frequencies and character sizes along transect T1 may suggest that evolutionary forces other than dispersal alone have left an imprint on the structure of the hybrid zone in this region.

The different patterns of changeover along the two transects also may be explained partly by the different spatial orientations of the two transects. We sampled along nonparallel transects for two reasons. First, because of the very restricted range of one species (*S. richteri*), transects across the hybrid zone in different areas must be nonparallel. Second, the patterns of variation expected along such transects are different for the two models of hybrid zone structure described above (tension zone and mosaic models; Rand and Harrison 1989). Our differently oriented transects therefore may allow us to determine which of these models best explains the patterns we observe. Indeed, the data suggest that the different orientations of the two transects can explain only the *degree* of mosaicism along the two transects: the existence of such mosaicism along both transects, regardless of their orientations, is inconsistent with the expectations of the tension zone model of hybridization.

In summary, the forces shaping the structure of the fire ant hybrid zone apparently are quite different over space and time, with historical patterns of dispersal and colonization important initially and other determinants such as differential fitness of genotypes due to intrinsic or extrinsic factors becoming more important with prolonged contact and interaction. However, it is difficult to infer the nature of the forces shaping the structure of this hybrid zone and, hence, the final outcome of hybridization between *S. invicta* and *S. richteri*, based only on the genetic structure along the two transects. Such inferences require additional data on the fitness of hybrids and the two parental species.

Fitness of Hybrids and Parental Species

Determination of the relative fitness of hybrids and the parental species is crucial for inferring the forces that structure a hybrid zone. We attempt to make inferences regarding the outcome of the genetic interactions between the two fire ant species and their hybrids by combining the present data with results from previous studies on the fitness of hybrids. Our genetic data suggest reduced fitness of individuals with hybrid genotypes. For example, we observed consistent departures of genotype proportions from HWE at our eight marker loci due to deficiencies of heterozygotes. Because heterozygotes at these loci generally are hybrids, the heterozygote deficiencies in the hybrid zone indicate that hybrids are strongly underrepresented compared to the frequency expected in a panmictic population with no selection. Consistent with this conclusion from analyses of single locus disequilibrium, we also observed a lack of hybrid individuals identified by their multilocus genotypes relative to the frequency expected with no selection. Finally, we observed significant linkage disequilibria between all pairs of loci surveyed within the hybrid zone, the expected outcome if strong selection favors parental combinations of genotypes and disfavors the recombinant multilocus genotypes characteristic of hybrids. Together these data are consistent with a reduction in fitness of individuals of mixed ancestry, such as may result from the breakup of coadapted gene complexes found in each of the parental species.

However, the observed patterns of deficiencies of single-locus and multilocus hybrid genotypes also are expected in a mosaic hybrid population comprising pure parental colonies intermingled with hybrids. The genetic structure along both transects suggests that this fire ant hybrid zone is indeed a mosaic hybrid zone and, most importantly, we found that single-locus and linkage disequilibria essentially disappeared when individuals with pure parental genotypes were eliminated from our analyses. The absence of linkage disequilibria when the parental genotypes are excluded is unexpected if the breakup of epistatic interactions among loci forming coadapted gene complexes is responsible for decreased hybrid fitness. Thus, the disequilibrium data alone are ambiguous for inferring the fitness of hybrids relative to the parental species.

On the other hand, the nonconcordant patterns of introgression between the two types of markers (genetic and morphological) clearly suggest reduced fitness of at least some hybrid genotypes. Differential introgression of markers has

been found in other hybrid zones (Patton et al. 1979; Harrison 1986, 1990; Bert and Harrison 1988; Dowling et al. 1989) and is expected only if some markers recombine more freely into a heterospecific background and, hence, flow more readily through a hybrid zone than others. If hybrids suffered no reductions in fitness (or if there were no resistance to the breakup of coadapted parental gene complexes), then all markers would recombine freely onto the genetic background of the other species and similar rates of introgression of all markers across the hybrid zone would be observed. Instead, all five morphological markers that we studied are impeded from flowing across the species' boundaries relative to the Mendelian genetic markers, a pattern that reflects the resistance of these polygenic characters to recombination and implies intrinsic fitness penalties to some individuals of mixed ancestry.

These data and previous data on the relative fitness of hybrids and the parental species are pertinent for inferring the eventual fate of the hybrid zone. One possible outcome of hybridization suggested by the earlier data is that *S. invicta* will replace ants with hybrid genotypes, as well as *S. richteri*, because of some intrinsic or extrinsic fitness advantage of the former species. Evidence for an intrinsic fitness advantage of *S. invicta* comes from a previous study in which significantly higher levels of fluctuating asymmetry of bilateral morphological characters were found in hybrids than in either parental species (Ross and Robertson 1990), suggesting some breakdown of coadapted parental gene complexes involved in the regulation of development in hybrids. This disruption of development presumably has detrimental effects on hybrid fitness. Levels of fluctuating asymmetry also differed between the parental species, with *S. invicta* having slightly lower levels than *S. richteri* (Ross and Robertson 1990). Thus, these data on fluctuating asymmetry suggest a hierarchy of fitness values related to intrinsic genetic factors, with pure *S. invicta* exhibiting the highest fitness and hybrids the lowest among the diverse genotypes present in the fire ant hybrid zone.

This difference in fitness between *S. invicta* and the other ants is suggested also by patterns of anomolous wing venation, the development of extra wing veins or the lack of whole or parts of veins, as described by Ross and Robertson (1990). Levels of these morphological abnormalities were elevated in hybrids and *S. richteri* compared to *S. invicta*, presumably indicating some breakdown in coordination of development that, again, carries a fitness penalty (Szymura and Barton 1986).

Another line of evidence for an intrinsic fitness advantage of *S. invicta* genotypes is the almost complete absence of *S. richteri* alleles in southern Mississippi and Alabama (Ross et al. 1987a; Ross and Robertson 1990; Shoemaker et al. 1994), a region where both *S. richteri* and hybrids were once quite common (Culpepper 1953; Buren et al. 1974; Vander Meer et al. 1985; Lofgren 1986). *Solenopsis richteri* was introduced into Mobile, Alabama prior to *S. invicta* and became well established throughout much of southern Alabama and Mississippi by the 1930s (Culpepper 1953; Buren et al. 1974; Lofgren 1986). However, following the introduction of *S. invicta* around 1935, also into Mobile, this second invader began to replace *S. richteri* throughout the southern part of the range until, by 1953, only morphologically identifiable *S. invicta* occurred over most of southern Alabama, Mississippi, and Georgia (Culpepper 1953). Ants identified morphologically as *S. richteri* were reported to occur at this time only in northern Mississippi, an area well ahead of the northerly expanding range of *S. invicta*. This remote area at the margin of the range of introduced fire ants is the only location where *S. richteri* is found today (Diffie et al. 1988; Shoemaker et al. 1994).

Solenopsis invicta has replaced hybrids as well as pure *S. richteri* as it has expanded its range northward. Studies of museum specimens using diagnostic biochemical markers (venom alkaloid and cuticular hydrocarbon patterns generated from gas chromatography) indicated that hybridization occurred as early as 1949 in southern Alabama, and it is reasonable to conclude that hybridization occurred even earlier wherever the two species came into contact (Lofgren 1986). Nonetheless, recent extensive genetic analyses of colonies from southern Mississippi and Alabama reveal that *S. richteri* alleles have now virtually disappeared from this area (see Fig. 1; Ross et al. 1987a; Ross and Robertson 1990; Shoemaker et al. 1994), as would be expected if *S. invicta* alleles conferred some fitness advantage in these areas.

The relative fitness of the parental species and hybrids may be influenced by extrinsic (environmental) factors as well as intrinsic factors. For instance, *S. richteri*, which has a more temperate distribution than *S. invicta* in South America (Trager 1991), may have a competitive advantage over *S. invicta* in the more northerly parts of the introduced range because of differences in the thermal biology of the two species (Buren et al. 1974; Ross et al. 1987a). An ecological advantage of *S. richteri* in cooler climates may offset its apparent intrinsic fitness disadvantages and allow it to persist in northern areas in the face of continued gene flow from *S. invicta*. Such differences in the relative fitness of the two species in different thermal environments also may explain the absence of *S. richteri* alleles throughout the southern parts of the range of fire ants in the United States.

Data on the fitness of hybrids ideally should include information on the relative fitnesses of the complete array of parental and recombinant genotypes under a diversity of environmental conditions. This is important because recent studies indicate that the fitness of different hybrid genotypes can be quite variable, ranging from lower to higher than that of their parental species (see Arnold and Hodges 1995). Thus, analyses that consider hybrids as a single class (such as above), rather than attempting to distinguish them on the basis of genetic constitution, are less likely to uncover the range of fitnesses of different types of hybrids.

We separated hybrids into different classes (i.e., *S. invicta*-like, *S. richteri*-like, and intermediate hybrids) to learn whether any decrease in hybrid fitness affects all hybrids uniformly or whether there are fitness disadvantages for only some hybrid classes. Most of the hybrid classes were represented in their expected proportions at all sites along the two transects, which suggests that a specific type of hybrid does not consistently suffer serious fitness breakdown. However, we did observe a general trend for *S. invicta*-like hybrids to be underrepresented at sites that have excesses of parental *S. invicta*, and the corresponding pattern for *S. richteri*-like hy-

brids and *S. richteri* (Appendices 4 and 5), suggesting that the fitness of the various hybrid classes varies in different regions of the hybrid zone and that hybrid fitness is affected by extrinsic selection associated with the presence of the most similar parental species. Thus, if slight differences exist between the parental species in the microhabitats in which they best perform, with *S. invicta* and *S. invicta*-like hybrids having similar optimal microhabitats, then reduced numbers of *S. invicta*-like hybrids at sites with an excess of *S. invicta* probably reflect a competitive advantage of the pure *S. invicta* genome over recombinant genomes with a strong *S. invicta* character. Other hybrids and *S. richteri* would be unaffected because of their presence in slightly different microhabitats. The same explanation would hold for the deficiency of *S. richteri*-like hybrids at sites where *S. richteri* predominates. Such microhabitat differences between the two parental species may involve degree of shading or of disturbance, which seem to influence the microdistribution of *S. invicta* and its congener *Solenopsis geminata* in Florida (Tschinkel 1987). If *S. richteri* is better adapted to cooler conditions, then the preferred habitats of the two species may overlap considerably in central Mississippi, forming a patchwork of optimal sites for the parental species and various hybrid genotypes that would promote the mosaic distributions we observed along our transects.

Conclusions

The distributional patterns along both transects are most consistent with the mosaic model of hybridization (Harrison 1986; Howard 1986; Rand and Harrison 1989), in which the distribution of the various fire ant genotypes initially is determined by historical patterns of colonization of uninhabited or newly available habitat patches. However, because of the recency of contact and dynamic nature of habitats in the study area, the patterns we observed may not reflect the equilibrium state of interactions between these two species and their hybrids. Indeed, both extrinsic and intrinsic selective forces may affect the fitness of the various fire ant genotypes and determine the eventual fate and structure of the hybrid zone. Several independent lines of evidence suggest that prolonged interactions may lead eventually to the replacement of *S. richteri* and hybrids by the intrinsically more fit *S. invicta* (Ross et al. 1987a; Ross and Robertson 1990; Shoemaker et al. 1994). On the other hand, *S. richteri* may persist in the northernmost parts of the introduced range because of extrinsically derived fitness advantages in cooler climates (Buren et al. 1974; Ross et al. 1987a), in which case the hybrid zone may persist over an area determined by the strength of intrinsic selection against hybrid genotypes (Key 1968; Barton and Hewitt 1985; Szymura and Barton 1986, 1991). Thus, because both stochastic and deterministic forces seem important in shaping the distributions of these fire ants, and because these forces apparently vary both spatially and temporally, the final outcome of contact and hybridization between *S. invicta* and *S. richteri* in the United States is difficult to predict. Future genetic studies of these same transects combined with detailed ecological and fitness studies of the two species and their hybrids may help in these predictive efforts.

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LITERATURE CITED

ARNOLD, M. L. 1992. Natural hybridization as an evolutionary process. Annu. Rev. Ecol. Syst. 23:237–261.

ARNOLD, M. L., AND S. A. HODGES. 1995. Are natural hybrids fit or unfit relative to their parents? Trends Ecol. Evol. 10:67–71.

AYALA, F. J., AND J. R. POWELL. 1972. Allozymes as diagnostic characters of sibling species of *Drosophila*. Proc. Nat. Acad. Sci. USA 69:1094–1096.

BARTON, N. H., AND K. S. GALE. 1993. Genetic analysis of Hybrid zones. Pp. 13–45 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

BARTON, N. H., AND G. M. HEWITT. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113–148.

———. 1989. Adaptation, speciation and hybrid zones. Nature 341:497–503.

BERT, T. M., AND R. G. HARRISON. 1988. Hybridization in western Atlantic stone crabs (genus *Menippe*): Evolutionary history and ecological context influence species interactions. Evolution 42: 528–544.

BULLINI, L. 1994. Origin and evolution of animal hybrid species. Trends Ecol. Evol. 11:422–426.

BUREN, W. F., G. E. ALLEN,, W. H. WHITCOMB, F. E. LENNARTZ, AND R. N. WILLIAMS. 1974. Zoogeography of the imported fire ants. J. N.Y. Entomol. Soc. 82:113–124.

CROWLEY, P. H. 1992. Resampling methods for computation-intensive data analysis in ecology and evolution. Annu. Rev. Ecol. Syst. 23:405–447.

CULPEPPER, G. H. 1953. Status of the imported fire ant in the southern states in July 1953. U.S. Dept. of Agriculture, Agricultural Research Administration, Bureau Entomology Plant Quarterly E-867.

DIFFIE, S., R. K. VANDER MEER, AND M. H. BASS. 1988. Discovery of hybrid fire ant populations in Georgia and Alabama. J. Entomol. Sci. 23:187–191.

DOBZHANSKY, T. 1940. Speciation as a stage in evolutionary divergence. Am. Nat. 74:312–321.

———. 1941. Genetics and the origins of species. Columbia Univ. Press, New York.

———. 1970. Genetics of the evolutionary process. Columbia Univ. Press, New York.

DOWLING, T. E., G. R. SMITH, AND W. M. BROWN. 1989. Reproductive isolation and introgression between *Notropis cornutus* and *Notropis chysocephalus* (family Cyprinidae): Comparison of morphology, allozymes, and mitochondrial DNA. Evolution 43: 620–634.

ECHELLE A. A., AND P. J. CONNOR. 1989. Rapid, geographically extensive genetic introgression after secondary contact between two pupfish species (*Cyprinodon*, Cyprinodontidae). Evolution 43:717–727.

HARRISON, R. G. 1986. Pattern and process in a narrow hybrid zone. Heredity 56:337–349.

———. 1990. Hybrid zones:Windows on evolutionary process. Pp. 69–128 *in* D. Futuyma and J. Antonovics, eds. Oxf. Surv. Evol. Biol. Oxford Univ. Press, New York.

———. 1991. Molecular changes at speciation. Annu. Rev. Ecol. Syst. 22:281–308.

———, ED. 1993. Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

HOCHBERG, Y. 1988. A sharper Bonferroni procedure for multiple tests of significance. Biometrika 75:800–802.

HOWARD, D. H. 1986. A zone of overlap and hybridization between two ground cricket species. Evolution 40:34–43

KEY, K. H. L. 1968. The concept of stasipatric speciation. Syst. Zool. 17:14–22.

KOCHER, T. D., AND R. D. SAGE. 1986. Further genetic analyses of a hybrid zone between leopard frogs (*Rana pipiens* complex) in central Texas. Evolution 40:21–33.

LOFGREN, C. S. 1986. History of imported fire ants in the United States. Pp. 36–49 *in* C. S. Lofgren and R. K. Vander Meer, eds. Fire ants and leaf cutting ants: Biology and management. Westview Press, Boulder CO.

LOFGREN, C. S., W. A. BANKS, AND B. M. GLANCEY. 1975. Biology and control of imported fire ants. Annu. Rev. Enotmol. 20:1–30.

LOUIS, E. J., AND E. R. DEMPSTER. 1987. An exact test for Hardy-Weinberg and multiple alleles. Biometrics 43:805–811.

MARKIN, G. P., J. H. DILLER, S. O. HILL, M. S. BLUM, AND H. R. HERMANN. 1971. Nuptial flight and flight ranges of the imported fire ant *Solenopsis saevissima richteri* (Hymenoptera: Formicidae). J. Ga. Entomol. Soc. 6:145–156.

MARKIN, G. P., H. L. COLLINS, AND J. H. DILLIER. 1972. Colony founding by queens of the red imported fire ant, *Solenopsis invicta*. Ann. Entomol. Soc. Am. 65:1053–1058.

MARKIN, G. P., J. H. DILLIER, AND H. L. COLLINS. 1973. Growth and development of colonies of the red imported fire ant, *Solenopsis invicta*. Ann. Entomol. Soc. Am. 66:803–808.

PATTON, J. L., J. C. HAFNER, M. S. HAFNER, AND M. F. SMITH. 1979. Hybrid zones in *Thomomys bottae* pocket gophers: Genetic, phenetic, and ecological concordance patterns. Evolution 33:860–876.

RAND, D. M., AND R. G. HARRISON. 1989. Ecological genetics of a mosaic hybrid zone: Mitochondrial, nuclear and reproductive differentiation of crickets by soil type. Evolution 43:432–439.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP. Vers. 1.2: A population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.

ROSS, K. G. 1993. The breeding system of the fire ant *Solenopsis invicta*: Effects on colony genetic structure. Am. Nat. 141:554–576.

ROSS, K. G., AND D. J. C. FLETCHER. 1985. Comparative study of genetic and social structure in two forms of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 17:349–356.

ROSS, K. G., AND J. L. ROBERTSON. 1990. Developmental stability, heterozygosity, and fitness in two introduced fire ants (*Solenopsis invicta* and *S. richteri*) and their hybrid. Heredity 64:93–103.

ROSS, K. G., AND J. C. TRAGER. 1990. Systematics and population genetics of fire ants (*Solenopsis saevissima* complex) from Argentina. Evolution 44:2113–2134.

ROSS, K. G., R. K. VANDER MEER, D. J. C. FLETCHER, AND E. L. VARGO. 1987a. Biochemical phenotypic and genetic studies of the two introduced fire ants and their hybrid (Hymenoptera: Formicidae). Evolution 41:280–293.

ROSS, K. G., E. L. VARGO, AND D. J. C. FLETCHER. 1987b. Comparative biochemical genetics of three fire ant species in North America, with special reference to the two social forms of *Solenopsis invicta* (Hymenoptera: Formicidae). Evolution 41:979–990.

ROSS, K. G., E. L. VARGO, AND D. J. C. FLETCHER. 1988. Colony genetic structure and queen mating frequency in fire ants of the subgenus *Solenopsis* (Hymenoptera: Formicidae). Biol. J. Linn. Soc. 34:105–117.

ROSS, K. G., E. L. VARGO, L. KELLER, AND J. C. TRAGER. 1993. Effect of a founder event on variation in the genetic sex-determining system of the fire ant *Solenopsis invicta*. Genetics 135: 843–854.

SHOEMAKER, D. D., J. T. COSTA, AND K. G. ROSS. 1992. Estimates of heterozygosity in two social insects using a large number of electrophoretic markers. Heredity 69:573–582.

SHOEMAKER, D. D., K. G. ROSS, AND M. L. ARNOLD. 1994. Development of RAPD markers in two introduced fire ants, *Solenopsis invicta* and *S. richteri*, and their application to the study of a hybrid zone. Mol. Ecol. 3:531–539.

SZYMURA, J. M., AND N. H. BARTON. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. Evolution 40: 1141–1159.

———. 1991. The genetic structure of the hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*: Comparisons between transects and between loci. Evolution 45:237–261.

TRAGER, J. C. 1991. A revision of the fire ants, *Solenopsis geminata* group (Hymenoptera: Formicidae: Myrmicinae). J. N.Y. Entomol. Soc. 99:141–198.

TSCHINKEL, W. R. 1987. Distribution of the fire ants *Solenopsis invicta* and *S. geminata* (Hymenoptera: Formicidae) in northern Florida in relation to habitat and disturbance. Ann. Entomol. Soc. Am. 81:76–81.

VANDER MEER, R. K., C. S. LOFGREN, AND F. M. ALVAREZ. 1985. Biochemical evidence for hybridization in fire ants. Fla. Entomol. 68:501–506.

WEIR, B. S. 1990. Genetic sata analysis. Sinauer, Sunderland, MA.

WHITHAM, T. G., P. A. MORROW, AND B. M. POTTS. 1991. Conservation of hybrid plants. Science 254:779–780.

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APPENDIX 1

Location of 44 collecting sites along transects T1 and T2 through the fire ant hybrid zone in central and eastern Mississippi. T1-0 and T2-0 represent the southernmost collecting sites along each of the two transects. All ants were collected within 0.5 kilometers of described location at every site.

Transect T1	
T1-0	Interstate Highway 55 (I-55) at mile marker 119 (Exit 119) near Canton, Mississippi.
T1-18	I-55 at mile marker 130, 3.0 miles south of Exit 133 (Vaughan, Mississippi).
T1-34	I-55 at mile marker 140, 1.0 mile north of Exit 139.
T1-50	I-55 at mile marker 150 (Exit 150).
T1-66	I-55 at mile marker 160, 4.0 miles north of Exit 156 (Durant, Mississippi).
T1-82	I-55 at mile marker 170, 4.0 miles south of Exit 174 (Vaiden, Mississippi).
T1-100	I-55 at mile marker 181, 4.0 miles south of the junction of I-55 and State Highway 82.
T1-106	Junction of I-55 (mile marker 185) and State Highway 82.
T1-109	I-55 at mile marker 187 (2.0 miles north of junction of I-55 and State Highway 82).
T1-114	I-55 at mile marker 190.
T1-119	I-55 at mile marker 193, 2.0 miles south of Exit 195 (Elliot, Mississippi).
T1-122	I-55 at mile markers 195 and 196 (junction of I-55 and State Highway 404 to Duck Hill, Mississippi).
T1-127	I-55 at mile marker 198.
T1-132	I-55 at mile marker 201, 5.0 miles south of Exit 206 (Grenada, Mississippi).
T1-135	I-55 at mile marker 203, 3.0 miles south of Exit 206 (Grenada, Mississippi).
T1-140	I-55 at mile marker 206 (Exit 206, Grenada, Mississippi).
T1-143	I-55 at mile marker 208, 2.0 miles north of Exit 206 (Grenada, Mississippi).
T1-148	I-55 at mile marker 211 (Exit 211, Hardy, Mississippi).
T1-153	I-55 at mile marker 214, 6.0 miles south of Exit 220 (Tillatoba, Mississippi).
T1-158	I-55 at mile marker 217, 3.0 miles south of Exit 220 (Tillatoba, Mississippi).
T1-161	I-55 at mile marker 219, 1.0 mile south of Exit 220 (Tillatoba, Mississippi).
T1-166	I-55 at mile marker 222, 2.0 miles north of Exit 220 (Tillatoba, Mississippi).
T1-183	I-55 at mile marker 233 (Exit 233, Enid, Mississippi).
T1-208	I-55 at mile marker 248 near Sardis, Mississippi.
Transect T2	
T2-0	State Highway 7 in Morgan City, Mississippi.
T2-24	State Highway 7 in Greenwood, Mississippi, 3.0 miles north of junction of State Highway 7 and State Highway 82.
T2-30	State Highway 7 ca. 0.8 miles north of Leflore/Carrol county line.
T2-35	State Highway 7 in Avalon, Mississippi.
T2-39	State Highway 7 in Leflore, Mississippi, ca. 1.3 miles north of Carrol/Grenada county line.
T2-45	State Highway 7, ca. 3.4 miles north of Leflore, Mississippi.
T2-50	State Highway 7 in Holcomb, Mississippi at junction of State Highway 7 and State Highway 8(35).
T2-58	State Highway 7(8) ca. 5 miles north of junction of State Highway 7 and State Highway 8(35) and 3 miles south of junction of State Highway 7 and I-55.
T2-64	I-55 at mile marker 208, 2.0 miles north of Exit 206 (Grenada, Mississippi).
T2-71	State Highway 7 ca. 2.2 miles north of junction of State Highway 7 and I-55.
T2-79	State Highway 7 ca. 1.5 miles north of Yalobusha/Grenada county line.
T2-86	State Highway 7 ca. 6.1 miles north of Yalobusha/Grenada county line and 1.4 miles south of Coffeeville, Mississippi.
T2-93	3.5 miles north of Coffeeville, Mississippi on highway connecting Coffeeville, Mississippi and Pine Valley, Mississippi.
T2-99	7.0 miles north of Coffeeville, Mississippi on highway connecting Coffeeville, Mississippi and Pine Valley, Mississippi.
T2-106	State Highway 32 in Pine Valley, Mississippi.
T2-114	5.0 miles East of Pine Valley, Mississippi and ca. 5.0 miles west of Banner, Mississippi.
T2-122	State Highway 9W in Banner, Mississippi.
T2-130	State Highway 9 at junction of State Highway 9 and County Road 174, Calhoun County.
T2-142	State Highway 9 ca. 2.0 miles north of Pontotoc/Calhoun county line near Randolph, Mississippi.
T2-169	State Highway 9 near Nixon, Mississippi, ca. 5.0 miles north of junction of State Highway 9 and State Highway 6.
T2-188	State Highway 9(348) in Ellistown, Mississippi.

APPENDIX 2

Frequencies of *S. invicta* alleles at allozyme and RAPD DNA markers at each site along transect T1 (site designations indicate distance in km from site T1-0). Values in parentheses represent 95% confidence intervals about the frequencies. Probabilities that observed genotype proportions at each site match those expected under Hardy-Weinberg equilibrium (HWE) are in brackets. All significant departures from HWE are due to a deficiency of heterozygotes and are indicated in bold; departures that remain significant when the significance levels are adjusted using a sequential Bonferroni procedure are denoted by asterisks. NT = no test.

Site	*N*	*Aat-2*	*Est-2*	*Est-4*	*G3pdh-1*	*Gpi*	*Odh*	*Pgm-1*	*UBC 105*
T1-0	44	0.94 (0.90–0.99) [1.000]	1.00 (1.00–1.00) NT	0.38 (0.34–0.53) [0.551]	0.48 (0.38–0.58) [1.000]	1.00 (1.00–1.00) NT	1.00 (1.00–1.00) NT	0.89 (0.81–0.94) [0.437]	—
T1-18	34	0.91 (0.84–0.97) [1.000]	1.00 (1.00–1.00) NT	—	0.45 (0.35–0.56) [0.300]	1.00 (1.00–1.00) NT	1.00 (1.00–1.00) NT	0.91 (0.85–0.97) [1.000]	—
T1-34	36	0.96 (0.92–1.00) [1.000]	1.00 (1.00–1.00) NT	0.43 (0.28–0.49) [1.000]	0.49 (0.38–0.58) [0.186]	1.00 (1.00–1.00) NT	1.00 (1.00–1.00) NT	0.90 (0.83–0.96) [1.000]	—
T1-50	28	0.91 (0.79–0.96) [0.347]	1.00 (1.00–1.00) NT	—	0.59 (0.30–0.52) [0.709]	1.00 (1.00–1.00) NT	1.00 (1.00–1.00) NT	0.91 (0.80–0.95) [1.000]	—
T1-66	33	0.88 (0.79–0.94) [1.000]	1.00 (1.00–1.00) NT	—	0.47 (0.35–0.56) [0.846]	1.00 (1.00–1.00) NT	1.00 (1.00–1.00) NT	0.89 (0.82–0.95) [1.000]	1.00 (1.00–1.00) NT
T1-82	31	0.94 (0.87–0.98) [1.000]	1.00 (1.00–1.00) NT	0.43 (0.29–0.56) [0.242]	0.37 (0.27–0.47) [0.132]	1.00 (1.00–1.00) NT	1.00 (1.00–1.00) NT	0.90 (0.82–0.98) [0.233]	1.00 (1.00–1.00) NT
T1-100	36	0.87 (0.79–0.94) [0.444]	0.90 (0.81–0.97) **[0.000]***	0.40 (0.28–0.53) **[0.037]**	0.26 (0.17–0.36) [1.000]	0.96 (0.89–1.00) **[0.042]**	0.90 (0.82–0.96) [0.290]	0.85 (0.76–0.92) [1.000]	0.90 (0.83–0.96) [1.000]
T1-106	35	0.70 (0.60–0.80) [0.449]	0.76 (0.63–0.87) **[0.016]**	0.26 (0.16–0.36) [0.402]	0.29 (0.19–0.40) [1.000]	0.69 (0.57–0.79) [0.700]	0.66 (0.54–0.79) [0.248]	0.77 (0.64–0.89) **[0.013]**	0.77 (0.67–0.87) [0.329]
T1-109	33	0.47 (0.32–0.62) **[0.038]**	0.53 (0.39–0.68) [0.098]	0.11 (0.05–0.18) [1.000]	0.23 (0.12–0.35) [0.313]	0.58 (0.45–0.71) [0.466]	0.56 (0.44–0.68) [0.176]	0.55 (0.41–0.68) [0.176]	0.53 (0.39–0.67) [0.294]
T1-114	33	0.45 (0.33–0.58) [0.730]	0.59 (0.48–0.70) [0.602]	0.22 (0.14–0.30) [0.295]	0.24 (0.14–0.35) [0.345]	0.56 (0.45–0.68) [1.000]	0.66 (0.53–0.79) [0.113]	0.55 (0.44–0.65) [0.498]	0.61 (0.50–0.71) [0.269]
T1-119	35	0.61 (0.49–0.74) [0.281]	0.71 (0.60–0.83) [0.151]	0.30 (0.20–0.40 [0.684]	0.28 (0.19–0.37) [0.388]	0.47 (0.34–0.61) **[0.040]**	0.61 (0.47–0.74) [0.139]	0.51 (0.39–0.63) [0.197]	0.57 (0.46–0.69) [1.000]
T1-122	41	0.75 (0.66–0.84) [0.678]	0.71 (0.57–0.83) **[0.000]***	0.18 (0.10–0.27) [0.578]	0.25 (0.16–0.35) [0.191]	0.60 (0.48–0.71) [0.190]	0.61 (0.48–0.72) **[0.043]**	0.59 (0.48–0.70) **[0.012]**	0.70 (0.59–0.80) [0.460]
T1-127	35	0.40 (0.27–0.53) [0.285]	0.63 (0.50–0.76) **[0.034]**	0.24 (0.16–0.33) [0.643]	0.21 (0.11–0.30) [1.000]	0.43 (0.30–0.54) [0.289]	0.44 (0.30–0.57) [0.068]	0.48 (0.36–0.61) [0.616]	0.53 (0.41–0.64) [1.000]
T1-132	32	0.40 (0.28–0.52) [1.000]	0.64 (0.52–0.77) [0.434]	0.37 (0.27–0.48) [1.000]	0.31 (0.20–0.42) [1.000]	0.50 (0.38–0.63) [1.000]	0.45 (0.31–0.58) [0.454]	0.61 (0.50–0.72) [1.000]	0.57 (0.41–0.73) **[0.002]***
T1-135	39	04.2 (0.32–0.53) [0.743]	0.66 (0.54–0.78) **[0.001]***	0.27 (0.17–0.38) [0.291]	0.22 (0.13–0.32) [0.458]	0.58 (0.47–0.69) [1.000]	0.70 (0.58–0.81) [0.089]	0.54 (0.41–0.65) [0.222]	0.54 (0.44–0.64 [1.000]
T1-140	33	0.50 (0.38–0.62) [1.000]	0.64 (0.52–0.76) [0.905]	0.24 (0.14–0.35) [0.345]	0.20 (0.09–0.30) [0.573]	0.44 (0.33–0.56) [1.000]	0.48 (0.36–0.61) [1.000]	0.53 (0.41–0.65) [0.591]	0.53 (0.41–0.65) [0.727]
T1-143	35	0.27 (0.14–0.41) **[0.000]***	0.37 (0.23–0.50) **[0.008]**	0.21 (0.10–0.31) [0.113]	0.10 (0.01–0.13) **[0.010]**	0.29 (0.17–0.41) **[0.012]**	0.26 (0.14–0.40) **[0.000]***	0.41 (0.29–0.54) **[0.003]**	0.23 (0.11–0.34) **[0.005]**
T1-148	30	0.37 (0.23–0.52) **[0.022]**	0.40 (0.27–0.53) [0.297]	0.17 (0.07–0.28) [0.154]	0.15 (0.07–0.23) [1.000]	0.30 (0.17–0.43) [0.073]	0.30 (0.18–0.42) [0.378]	0.47 (0.32–0.62) [0.074]	0.25 (0.12–0.38) **[0.004]**
T1-153	32	0.35 (0.23–0.47) [1.000]	0.45 (0.33–0.59) [0.122]	0.22 (0.11–0.34) [0.101]	0.22 (0.14–0.31) [0.287]	0.45 (0.31–0.58) [0.481]	0.56 (0.42–0.70) [0.131]	0.60 (0.47–0.72) [0.888]	0.44 (0.33–0.53) [0.277]
T1-158	32	0.42 (0.31–0.53) [0.707]	0.52 (0.39–0.64) [0.515]	0.17 (0.09–0.25) [0.563]	0.16 (0.08–0.25) [0.564]	0.56 (0.42–0.70) [0.172]	0.59 (0.47–0.72) [1.000]	0.59 (0.47–0.72) [0.554]	0.40 (0.28–0.55) [1.000]